REMARKS

I. Preliminary Comments

Applicants note with gratitude that the Examiner has withdrawn certain of the previous rejections. These include:

(1) The indefiniteness rejection of claims 70-226 under 35 U.S.C. § 112 (second paragraph);

(2) The Section 102 anticipation rejection based on Zhang U.S. 6,410,010 of claims 72, 74, 89 (sic 79)-82, 84 and 101-226;

(3) The Section 103 obviousness rejection over Zhang '010 in combination with Shabram, U.S. 5,837,520 of each of claims 73-74, 77, 104-105, 108, 135-136, 139, 166-167, 170, 197-198 and 201; and

(4) The written description rejections of claims 70-226 under 35 U.S.C. § 112, first paragraph, with respect to the recitation of "any promoter" and "any gene."

(5) In addition, the provisional obviousness-type double patenting rejection over copending application Serial No. 09/203,078 was held in abeyance.

Claims 78, 109, 140, 171 and 202 directed to therapeutic compositions "having a BSA content below the detection level of a western blot assay" have been cancelled and those limitations introduced into each of independent claims 70, 101, 132, 163 and 194.

In addition a number of dependent claims have now been rewritten in independent form to incorporate these and other limitations. In addition, claims 103, 134, 165, 196 have been amended to place them in independent form as well as to correct a typographical error made in the Preliminary Amendment of December 17, 2001 wherein "purified" was omitted from the recitation of "a substantially therapeutic adenovirus composition." Each of these amendments is supported in the original disclosure and no new matter is introduced hereby.

It is submitted that these amendments distinguish over the art of record and otherwise place all of the pending claims in condition for allowance.

II. The Outstanding Rejections

As discussed above, the Examiner withdrew a number of the previous rejections. In addition, the Examiner maintained, and in some cases elaborated upon the following rejections:

(6) Claims 70-226 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 12 and 31 of U.S. Patent 6,726,907.

(7) The written description rejections of claims 70-226 remain rejected under 35 U.S.C. §112, first paragraph, relating to "a therapeutic adenovirus" was maintained.

(8) The lack of enablement rejection of claims 70-226 are rejected under 35 U.S.C. §112, first paragraph, was maintained for a list of reasons extending from pages 7-25 of the Office Action.

(9) While the Examiner withdrew the Section 102 rejection from claims 72, 74, 79-82, 84 and 101-226 as discussed above, he maintained the rejection of claims 70-71, 73, 75-77, 83 and 85-100 under 35 U.S.C. §102(e) as being anticipated by Zhang et al., '010 as further evidenced by Huyghe, et al., Human Gene Therapy, 6:1403-1416 (1995).

Further, a number of new rejections were entered as set out below:

(10) Claims 70-226 are newly rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-89 of U.S. Patent 6,194,191.

(11) , Claims 70, 78-82 and 84 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhang et al. as further evidenced by Huyghe et al. as applied to claim 70 above, and further in view of Perrin et al., (1995) Vaccine, 13(13): 1244-50.

(12) Claim 74 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhang et al. as further evidenced by Huyghe et al. and Nadeau et al., (1996) Biotechnology and Bioengineering, 51:613-623, or Trepanier et al., (1981) J. Virological Methods, 3: 201-11.

(13) Claims 101, 103-104 and 106-131 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhang et al. as further evidenced by Huyghe et al as applied to claims 70-71, 73, 75-77, 83 and 85-100 above, and further in view of Perrin et al., (1995) Vaccine, 13(13): 1244-50.

(14) Claims 132, 134-135 and 137-162 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhang et al. as further evidenced by Huyghe et al. and Perrin et al.

(15) Claim 105 is rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al. as further evidenced by Huyghe et al. and further in view of Perrin et al. as applied to claim 101 above, and further in view of Nadeau et al. or Trepanier et al.

(16) Claims 163, 165-166, 168-170, 176 and 178-198 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhang et al. as further evidenced by Huyghe et al. and in view of Graham et al, (1991) Methods in Molecular Biology, Vol. 7, Ed. By Murray, published by Humana Press, In., Clifton, NJ., pp. 109-128.

(17) Claims 171-175 and 177 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhang et al. as further evidenced by Huyghe et al. and Graham et al. as applied to claim 163 above, and further in view of Perrin et al.

(18) Claim 167 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhang et al. as further evidenced by Huyghe et al. and in view of Graham et al., and further in view of Nadeau et al. or Trepanier.

(19) Claims 194, 196-197, 199-201, 207 and 209-226 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhang et al. and Huyghe et al. as further evidenced by Huyghe et al.

(20) Claims 194, 202-206 and 208 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhang et al. and Huyghe et al. as further evidenced by Huyghe as applied to claim 194 above, and further in view of Perrin et al.

(21) Claim 198 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Zhang et al. and Huyghe et al. as further evidenced by Huyghe, and further in view of Nadeau et al. or Trepanier.

Significantly, a number of claims were indicated to be free of the prior art (although not of obviousness-type double patenting over U.S. 6,194,191). Those claims are as follows:

> 72. (Previously Presented) The method of claim 70, wherein the therapeutic adenovirus comprises a substantially purified therapeutic adenovirus composition.
>
> 102. (Previously Presented) The method of claim 101, wherein the therapeutic adenovirus comprises 70% +/- 10% of the starting PFU of the lysate of step iv.
>
> 133. (Previously Presented) The method of claim 132, wherein the therapeutic adenovirus composition comprises 70% +/- 10% of the starting PFU of the lysate of step iv.
>
> 136. (Previously Presented) The method of claim 132, wherein the therapeutic adenovirus composition has a contaminating nucleic acid concentration of less than 0.2 ng/ml.
>
> 164. (Previously Presented) The method of claim 163, wherein the therapeutic adenovirus composition comprises 70% +/- 10% of the starting PFU of the lysate of step iv.

195. (Previously Presented) The method of claim 194, wherein the therapeutic adenovirus composition comprises 70% +/- 10% of the starting PFU of the lysate of step iv.

III. Patentability Arguments

A. The Obviousness-Type Double Patenting Rejection Should Be Withdrawn

The rejection of claims 70-226 under the judicially created doctrine of obviousness-type double patenting over claims 12 and 31 of Zhang et al., parent patent U.S. 6,726,907 and claims 1-89 of Zhang et al., grandparent patent U.S. 6,194,191 should be withdrawn in light of the terminal disclaimer submitted herewith accompanied by the appropriate fees.

B. The Provisional Double Patenting Rejection Should Remain Deferred.

The provisional rejection of claims 70-226 under the judicially created doctrine of obviousness-type double patenting over Zhang et al., Copending Application No. 09/203,078 (US 2004/0229335 A1) should be deferred because the pending claims in that case have not yet been patented. Accordingly, it is not necessary to take any action with respect to them at this time.

C. The Rejections for Lack of Written Description Should Be Withdrawn.

The rejections of claims 70-226 for lack of written description under 35 U.S.C. §112 (first paragraph) should be withdrawn because the claims serve as their own written description and they make clear that the inventors were in possession of the invention so claimed at the time their application was filed. (See *In re Koller*, 204 USPQ 702 (CCPA 1980)) Specifically, while originally filed claim 5 recited "an adenovirus which comprised an adenoviral vector encoding an exogenous gene construct" that claim depended from independent claim 1 which required no such limitation. Moreover, Applicants describe their invention throughout their disclosure and teach its practice in a manner such that those of ordinary skill would recognize that Applicants were in possession of the invention claimed.

The suggestion that the application lacks written descriptive support beyond a therapeutic adenovirus comprising transgene and promoter element is incorrect because the application does not describe such a limitation and the Summary of the Invention states that

"an adenoviral vector encoding an exogenous gene construct" is "a preferred embodiment." (Page 4, lines 22-23).

Moreover, those of skill in the art at the time the first priority application was filed (November 20, 1996) would have recognized that the therapeutic adenovirus compositions were not limited to those which comprise a transgene. For example, ONYX-015 (Onyx Pharmaceuticals, Emeryville, CA) is an E1B-55kD gene-deleted adenovirus in which is engineered to selectively replicate in and lyse p53-deficient cancer cells. (See, Bischoff et al. "An adenovirus mutant that replicates selectively in p53-deficient human tumor cells." Science 274: 373-376 (1996) attached hereto at Exhibit A). This type of therapeutic adenovirus composition does not comprise or require a transgene. Nevertheless, methods of treating a patient with the adenovirus composition would benefit from its purification.

The "essential goal" of the written description requirement is to clearly convey that "an applicant has invented the subject matter which is claimed" (*In re Barker*, 194 USPQ 470, 473 (CCPA 1977) cited in MPEP 2163) but no reasons have been set forth why one of ordinary skill would not believe that Applicants were in possession of their originally claimed invention of treating patients with purified therapeutic adenovirus compositions at the time of filing. In contrast with the lack of rationale why those of ordinary skill would not believe Applicants were in possession of the invention described in their claims, there remains a "strong presumption" that an adequate written description is present in the specification as filed (MPEP 2163 II A citing *In re Wertheim* 191 USPQ 90, 96 (CCPA 1976)). The MPEP further states that "the rejection of an original claim for lack of written description should be rare."

To the extent that the issues raised in the outstanding "written description" rejection are really issues relating to the enablement requirement of Section 112 (first paragraph) the Examiner's attention is directed to the later discussion directed to those issues.

D. <u>The Rejections for Lack of Enablement Should Be Withdrawn.</u>

The rejections for lack of enablement should be withdrawn because the specification provides ample direction to practice the claimed invention beyond just practice of gene therapies already known to be successful with adenoviral vectors. With respect to this rejection, the claimed invention is generally directed to "A method of treating a patient with a

therapeutic adenovirus composition" which preparation has been prepared by a specifically recited process.

The specification enables preparation of the therapeutic compositions and practice of the methods of the claims beyond just known gene therapy methods. Applicants respectfully note that "it is incumbent upon the Patent Office... to explain *why* it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement." MPEP 2164.05 (quoting *In re Marszocchi*, 439 F.2d 220, 224, 169 U.S.P.Q. 367, 370 (CCPA 1971)). As will be discussed below, the reasons expressed in the Action fail to cast doubt as to the sufficiency of the present disclosure to allow a person of ordinary skill in the art to practice the invention without undue experimentation. There is no reason why a person could not take the disclosure and practice the invention as claimed in claims 70-226. Accordingly, the specification teaches how to make and use the claimed invention, and thus, it enables claims 70-226.

While the Action argues that gene therapy is difficult and unpredictable and that these unpredictabilities have not yet been overcome by the art Applicants point out that the PTO is required to assume that the specification complies with the enablement provisions of Section 112 unless it has "acceptable evidence or reasoning" to suggest otherwise. *In re Marszocchi*, 439 F.2d 220, 224, 169 U.S.P.Q. 367, 370 (CCPA 1971) With respect to those specific contentions, the Action provides no such evidence to challenge the specification's assertion that a method of treatment would be effected. For example, the specification teaches that tumor cells may be treated and the claims do not limit tumor cells to those lacking p53. This must be taken at face value unless the Action can point to evidence that undermines this assertion.

With respect to cancer treatment, there are references that indicate the invention will work as claimed; these references indicate that tumor cells carrying a wild-type p53 gene are susceptible to p53 gene therapy. For example, the reference of Hamada *et al.* Cancer Research 56: 3047-3054 (1996) cited previously shows that a number of p53-positive cervical cell lines -HeLa, C4-I, MS751, ME180, CaSki, and SiHa-are amenable to p53 gene therapy using adenovirus. This reference addresses the issue of whether cancers with mutations unrelated to p53 may be treated by the claimed invention. Thus, contrary to the unsupported assertion in the Action, there is no reason to doubt the invention will work as claimed with

respect to cancer. Further, the specification teaches that other disease conditions may be treated by adenoviral administration of other therapeutic genes. As discussed below, the specification teaches treatment of adenosine deaminase deficiency, human blood clotting factor IX deficiency in hemophilia B, cystic fibrosis, involving the replacement of the cystic fibrosis transmembrane receptor gene as well as treatment of hyperproliferative disorders such as rheumatoid arthritis or restenosis by transfer of genes encoding angiogenesis inhibitors or cell cycle inhibitors.

The Action urges that Applicants review the references Deonarain, Verma, Eck et al., Gorecki and Green to show that gene therapy is unpredictable. First, these references only generalize about gene therapy. More importantly, none of the references state that gene therapy will not work and is a complete failure. Instead, they focus on more clinical issues, which are above and beyond the standards for patentability. See *In re Krimmel*, 292 F.2d 948, 954 (C.C.P.A. 1961) ("There is nothing in the patent statute or any other statues...which give the Patent Office the right or the duty to require an applicant to prove that compounds or other materials which he is claiming, and which he has state are useful for 'pharmaceutical applications,' are safe, effective, and reliable for use with humans.").

Deonarain identifies the issues of targeting genes to sufficient populations of cells and of adequate expression but does not state that gene therapy will not work. In fact, Applicants' own evidence and that of others demonstrates (and the Examiner accepts) that gene therapy does work with respect to the p53 gene and cancer. Accordingly, the issues raised by Dornarain do not indicate that the specification is defective in any way.

The text of the Verma reference makes no comment about Ad-p53 cancer therapy that would suggest that the claims are not enabled. Applicants emphasize that the claims are directed to Ad-p53 cancer therapy and other gene therapies, which the specification teaches, and this reference does not indict the disclosure in any way. For example, the reference does not say that practicing cancer or other gene therapies according to the claims would require undue experimentation. Accordingly, Applicants submit that the Verma reference is not dispositive on the issue of enablement.

Similarly, the Eck reference identifies a number of factors important to gene therapy but does not suggest that those of skill in the art would be unequipped to address such factors in a gene therapy protocol. Gorecki addresses similar issues of stability and of expression

levels but does not state that such are obstacles which are insurmountable to those of ordinary skill in the art.

Finally, the Action contrasts the identification by Green of key hurdles to be overcome for effective gene therapy with adenoviral vectors and cancer with the disclosure of Zhang U.S. 6,740,320 which describes those very hurdles being overcome in the use of p53-encoding adenoviruses for treating cancer in animals. This being the case, there exists no reason to doubt the enablement of the claimed invention by Applicants' specification.

Applicants further note that there are other scientific articles that rebut any contention that intravenous or systemic delivery of adenovirus vectors will not work. The article of Nemunaitis et al. Gene Therapy 8:746-759 (2001) cited previously indicates that "intravenous administration of genetically altered adenovirus is a feasible approach." In Nemunaitis et al., the authors report their findings from a dose-escalation clinical trial involving patients with different cancers and they confirmed intratumoral delivery and replication of the adenovirus. See Nemunaitis et al. at 749, Table 1, and Fig. 3 and 4. In another article Shiriwawa et al., Cancer Gene Therapy 5 (5): 274-280 (1998) cited previously mice were treated for osteosarcoma pulmonary metastasis using an intravenously administered adenovirus containing a thymidine kinase gene. Accordingly, the Action's contention that alternative delivery methods are problematic is rebutted.

The specification provides a teaching that allows a person of ordinary skill in the art to practice the invention without undue experimentation, and none of the references cited by the Action provide evidence that the invention will not work as described and claimed. More particularly, Applicants' disclosure provides examples of diseases other than cancer for which the viral vectors of the invention would be useful including, but not limited to, adenosine deaminase deficiency, human blood clotting factor IX deficiency in hemophilia B, and cystic fibrosis, which would involve the replacement of the cystic fibrosis transmembrane receptor gene. The disclosure also teaches vectors that could be used for treatment of hyperproliferative disorders such as rheumatoid arthritis or restenosis by transfer of genes encoding angiogenesis inhibitors or cell cycle inhibitors. The specification further teaches that transfer of prodrug activators such as the HSV-TK gene could be used in the treatment of hyperproliferative disorders, including cancer. (Page 47, line 25 through page 48, line 3)

Applicants' disclosure also teaches the use of adenoviral vectors comprising genes encoding enzymes such as cytosine deaminase, hypoxanthine-guanine phosphoribosyl-

transferase, galactose-l-phosphate uridyltransferase, phenylalanine hydroxylase, glucocerbrosidase, sphingomyelinase, alpha-L-iduronidase, glucose-6-phosphate dehydrogenase, HSV thymidine kinase and human thymidine kinase. (Page 46, line 27 through page 47, line 2) The specification further teaches adenoviral vectors comprising genes encoding hormones including growth hormone, prolactin, placental lactogen, luteinizing hormone, follicle-stimulating hormone, chorionic gonadotropin, thyroid-stimulating hormone, leptin, adrenocorticotropin (ACTH), angiotensin I and II, beta-endorphin, beta-melanocyte stimulating hormone (beta-MSH), cholecystokinin, endothelin I, galanin, gastric inhibitory peptide (GIP), glucagon, insulin, lipotropins, neurophysins, somatostatin, calcitonin, calcitonin gene related peptide (CGRP), beta-calcitonin gene related peptide, hypercalcemia of malignancy factor (1-40), parathyroid hormone-related protein (107-139) (PTH-rP), parathyroid hormone-related protein (107-111) (PTH-rP), glucagon-like peptide (GLP-1), pancreastatin, pancreatic peptide, peptide YY, PHM, secretin, vasoactive intestinal peptide (VIP), oxytocin, vasopressin (AVP), vasotocin, enkephalinamide, metorphinamide, alpha melanocyte stimulating hormone (alpha-MSH), atrial natriuretic factor (5-28) (ANF), amylin, amyloid P component (SAP-1), corticotropin releasing hormone (CRH), growth hormone releasing factor (GHRH), luteinizing hormone-releasing hormone (LHRH), neuropeptide Y, substance K (neurokinin A), substance P and thyrotropin releasing hormone (TRH). Also disclosed by the specification is the use of adenoviral vectors comprising genes encoding interleukins and cytokines including Interleukin 1 (IL-1), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11 IL-12, GM-CSF and G-CSF. (Page 47, lines 4-23)

The specification also teaches the use of the adenoviral vectors as vaccines for the administration of genes encoding antigens such as viral antigens, bacterial antigens, fungal antigens or parasitic antigens. Such antigens are disclosed to include those from viruses such as picomavirus, coronavirus, togavirus, flavirviru, rhabdovirus, paramyxovirus, orthomyxovirus, bunyavirus, arenvirus, reovirus, retrovirus, papovavirus, parvovirus, herpesvirus, poxvirus, hepadnavirus, and spongiform virus. Preferred viral targets include influenza, herpes simplex virus 1 and 2, measles, small pox, polio or HIV. Pathogens include trypanosomes, tapeworms, roundworms, helminths. Genes encoding other antigens include those encoding tumor markers, such as fetal antigen or prostate specific antigen, as well as genes encoding HIV *env* proteins and hepatitis B surface antigen. (See page 50, lines 5-13)

With respect to treatment of cancer, the specification teaches administration of adenoviral vectors comprising genes including $p16^{b}$, $p21^{WAF1, CIP1, SDI1}$ and $p27^{KIP1}$ as well as other tumor suppressors such as RB, APC, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, zac1, p73, BRCA1, VHL, FCC, MMAC1, MCC, p16, p21, p57, C-CAM, p27 and BRCA2. The specification further teaches treatment of cancer by administration of adenoviral vectors comprising genes encoding inducers of apoptosis, such as Bax, Bak, Bcl-X_S, Bik, Bid, Harakiri, Ad E1B, Bad and ICE-CED3 proteases. (See page 46, lines 22-24) The specification also teaches that adenoviral vectors of the invention can comprise antisense nucleic acids which are complementary to the base sequences of oncogene-encoding DNA and RNA such as *ras, myc, neu, raf erb, src, fms, jun, trk ret, gsp, hst, bcl* and *abl*. (Page 48, lines 6-7)

Applicants' specification also enables those of skill in the art to practice administer adenoviral vectors by a variety of modes. Specifically, pages 72-74 of the disclosure teach that the viral particles of the invention may be administered by a variety of therapeutic regimens so long as the target tissue is available via that route. Suitable routes include oral, nasal, buccal, rectal, vaginal and topical administration. The disclosure further teaches that administration can be by orthotopic, intradermal subcutaneous, intramuscular, intraperitoneal, or intravenous injection. Pharmaceutical compositions of the invention would normally include physiologically acceptable carriers, buffers or other excipients. The specification teaches that for application against tumors, direct intratumoral injection, injection of a resected tumor bed, regional (i.e., lymphatic) or general administration can be practiced as well as continuous perfusion over hours or days via a catheter to a disease site, e.g., a tumor or tumor site.

The disclosure teaches that the therapeutic compositions of the invention may be administered in the form of injectable compositions either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. These preparations also may be emulsified. A typical composition for such purpose comprises a pharmaceutically acceptable carrier such as human serum albumin in phosphate buffered saline. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous carriers are disclosed as including

water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose and the like. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial agents, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components the pharmaceutical composition are adjusted according to well known parameters.

The specification also teaches formulations suitable for oral administration. Oral formulations include such typical excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. The compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders. When the route is topical, the form may be a cream, ointment, salve or spray. Those of skill in the art would appreciate that an effective amount of the therapeutic agent is determined based on the intended goal, for example (i) inhibition of tumor cell proliferation, (ii) elimination or killing of tumor cells, (iii) vaccination, or (iv) gene transfer for long term expression of a therapeutic gene.

Applicants previously submitted the declaration of Kerstin B. Menander, Ph.D., M.D. the Vice President of Clinical Development at Introgen Therapeutics ("Introgen") as evidence that the rejected claims are enabled. Dr. Menander's declaration set forth the numerous clinical trials, involving Introgen's INGN 201 adenovirus-p53 composition, which is disclosed in the specification of the present application, that are underway or have recently been completed or that have been approved. The Declaration also set forth a number of clinical trials that have employed another adenoviral p53 construct, Schering Plough's SCH 58500 adenovirus-p53 construct. It further describes the clinical trials of other researchers beyond the field of cancer therapeutics including for treatment of cystic fibrosis, partial ornithine transcarbamylase deficiency, Canavan Disease, Peripheral Vascular Disease, Critical Limb Ischemia, Diabetic Ulcers, Coronary Artery Disease, Peripheral Arterial Occlusive Disease, Hemophilia, Treatment of Dialysis Patients, and Parkinson's Disease.

Applicants further point to the U.S. Patent and Trademark Office's own training guide, albeit on utility instead of enablement, which states, "[A]s a general rule, if an applicant has initiated human clinical trials for a therapeutic product or process for a therapeutic or process, Office personnel should presume that the applicant has established that the subject matter of that trial is reasonably predictive of having the asserted therapeutic utility." MPEP 2107.03 IV. Because the requirements for utility and enablement are

intertwined, Applicants contend that the submission of bountiful clinical trial evidence strongly weighs against a rejection for enablement. The clinical trial evidence shows that adenovirus-p53 is being tested against a number of cancers, including head and neck, non-small cell lung carcinoma, ovarian, breast, esophageal, lung, glioma, prostate, bladder, and solid tumors form colon cancer, breast cancer, prostate cancer, sarcomas, non-small cell lung carcinomas and head and neck cancer. This evidence further indicates that administration of Ad-p53 is achieved regionally, intravenously, directly, intraperitoneally, and intravesically. The Declaration of Dr. Menander confirms the enablement of the claimed invention. Applicants respectfully request that the rejection of the claims for lack of enablement be withdrawn in view of the following reasons.

E. The Anticipation Rejection of Claims 70-71, 73, 75-77, 83 and 85-100 Over Zhang et al., '010 Should Be Withdrawn.

The anticipation rejection of claims 70-71, 73, 75-77, 83 and 85-100 Over Zhang et al., '010 "as evidenced by Huyghe" should be withdrawn for the reasons set out previously and further in light of the amendment of the independent claims to recite that the adenovirus composition has a BSA content below the detection level of a western blot assay. As acknowledged by the Examiner, Zhang fails to teach BSA levels below the detection limit of western blots and Huyghe fails to make up for that deficiency in Zhang. Accordingly, the anticipation rejection of claims 70-71, 73, 75-77, 83 and 85-100 should be withdrawn.

F. Claim 72 is in Independent Form and Should be Allowed.

Claim 72, which was previously indicated to be allowable over the art has been placed in independent form and should now be allowed.

G. The Obviousness Rejection of Claims 70 and 78-82 and 84; Claims 101, 103-104 and 106-131; Claims 132, 134-135 and 137-162; and Claims 194, 196-197, 199-206 and 208-226 Over Zhang et al., '010 and Huyghe In View of Perrin Should Be Withdrawn.

The obviousness rejection of claims 70 and 78-82 and 84, of claims 101, 103-104 and 106-131, of claims 132, 134-135 and 137-162 and claims 194, 196-197, 199-206 and 208-226 over Zhang et al., as evidenced by Huyghe and further in view of Perrin should also be withdrawn for the reasons set out above with respect to Zhang and Huyghe. These reasons

include the failure of those references to suggest an adenovirus composition which "has a BSA content below the detection level of a western blot assay."

Perrin, however, fails to make up for the deficiencies in Zhang and Huyghe with respect to how serum-free media could be used in the context of preparing adenovirus compositions because Perrin teaches the use of serum-free media in the context of a rabies virus system which is quite distinct from an adenovirus system! While Perrin reports that a rabies virus can be raised in a host cell grown in serum-free media, Perrin fails to instruct whether, much less how, adenovirus host cells could be cultured to successfully produce adenovirus compositions in the absence of serum. In the absence of some more relevant and specific teaching, the rejections over the combination of Zhang, Huyghe and Perrin should accordingly be withdrawn.

H. The Obviousness Rejections of Claims 74, 105, 167 and 198 Over Zhang et al., '010 and Huyghe In View of Nadeau and Trepanier Should Be Withdrawn.

The obviousness rejections of claims 74, 105, 167 and 198 over Zhang et al., as evidenced by Huyghe and further in view of Nadeau and Trepanier should also be withdrawn because these claims depend from claims 70, 101, 163 and 194 which themselves are patentable over Zhang and Huyghe for the reasons set out above. Further, the fact that Applicants have established that their method is capable of achieving a contaminating nucleic acid level of less than 0.8 ng/ml (as disclosed in Table 10 in the specification as cited in the Action) is a hindsight observation and may not be applied to render the claims obvious. The hindsight observation that Applicants' method actually works does nothing to establish that Nadeau and Trepanier would have led one of ordinary skill in the art to believe beforehand that such a result could be achieved.

I. The Rejection of Claims 163, 165-170, 172-198 Over Zhang As Evidenced by Huyghe and In View of Graham under 35 U.S.C. §103(a) Should Be Withdrawn.

The rejections of claims 163, 165-170, 176 and 178-198 based upon Zhang, and Huyghe and further in view of Graham should be withdrawn for the reasons set out above and because Graham, which discloses non-freeze-dried methods for lysis, does not otherwise

remedy the deficiencies of Zhang and Huyghe as already set out in Section E above. Further, independent claim 163 has now been amended to incorporate the limitation of claim 171 (now cancelled) which has a BSA content below the detection level of a western blot assay. Accordingly, the rejection under Zhang, Huyghe, Graham and Perrin should not be entered against the claims because Perrin is directed to rabies virus and fails to teach how serum-free media could be used in the context of preparing adenovirus compositions as discussed above.

CONCLUSION

In view of the above amendment, applicants believe the pending application is in condition for allowance.

Respectfully submitted,

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An Adenovirus Mutant That Replicates Selectively in p53-Deficient Human Tumor Cells

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The human adenovirus *E1B* gene encodes a 55-kilodalton protein that inactivates the cellular tumor suppressor protein p53. Here it is shown that a mutant adenovirus that does not express this viral protein can replicate in and lyse p53-deficient human tumor cells but not cells with functional p53. Ectopic expression of the 55-kilodalton E1B protein in the latter cells rendered them sensitive to infection with the mutant virus. Injection of the mutant virus into p53-deficient human cervical carcinomas grown in nude mice caused a significant reduction in tumor size and caused complete regression of 60 percent of the tumors. These data raise the possibility that mutant adenoviruses can be used to treat certain human tumors.

Deletion or mutation of the p53 tumor suppressor gene occurs frequently in most types of human cancer (*1*). Tumors lacking functional p53 are, in many cases, refractory to chemotherapy or radiation (*2*). It is therefore critically important to develop therapeutic strategies to treat p53-deficient tumors.

DNA tumor viruses such as adenoviruses infect quiescent cells and induce them into the S phase of the cell cycle so that viral DNA replication can proceed (*3*). The E1A protein of human adenoviruses, which binds pRB, p300, and other related proteins, is largely responsible for this forced entry into the S phase (*4*). The *E1B* region of the viral genome encodes a 55-kD protein (E1B 55K) that binds and inactivates p53 (*5*). This binding is essential to virus replication, possibly because E1A induces p53-dependent apoptosis. We hypothesized that an adenovirus mutant that does not produce E1B 55K should be unable to replicate in normal cells but would be able to replicate in cells lacking functional p53. The dl1520 virus is such a mutant. This human group C adenovirus contains an 827–base pair deletion in the *E1B* region and a point mutation at codon 2022 that generates a stop codon preventing expression of a truncated protein from the deleted gene (*6*). The *E1B 19K* gene, whose protein product suppresses apoptosis, is not affected by this deletion.

We first tested the ability of this virus to grow in cells lacking functional p53. The dl1520 virus grew as efficiently as wild-type adenovirus in C33A cervical carcinoma cells, which express p53 with an inactivating mutation at codon 273 (*7*) (Fig. 1A). Under identical conditions, dl1520 grew poorly in U20S osteocarcinoma cells, which retain wild-type p53 (*8*), producing about 100 times less infectious virus than did wild-type adenovirus. Polymerase chain reaction procedures (*9*) were used to verify that the virus produced by dl1520-infected C33A cells retained the *E1B* deletion and that the infection was not due to wild-type adenovirus contamination (*9*).

We also performed cytopathic effect (CPE) assays on a panel of human tumor cells and normal cells infected with either dl1520 or wild-type adenovirus. The dl1520 virus had no detectable cytopathic effect on normal human diploid fibroblasts or on tumor cells retaining wild-type p53, whereas wild-type adenovirus caused compete lysis under identical conditions (Fig. 1B). In contrast, both dl1520 and wild-type adenovirus killed C33A cells with high efficiency. This analysis was extended to a variety of tumor cell lines of known p53 status (*10*), including four cervical carcinoma cell lines expressing human papillomavirus (HPV) E6, which inactivates p53 through ubiquitin-mediated protein turnover (*7*); four colon carcinoma cell lines containing different mutant forms of p53; U373 glioblastoma cells (codon 273 mutation) (*11*); and HS700T pancreatic adenocarcinoma cells (codon 249 mutation) (*12*). In each case, dl1520 killed cells with an efficiency comparable to that of wild-type adenovirus (*10*).

These results show a clear correlation between p53 status and susceptibility to dl1520 and are consistent with our original hypothesis. However, the dl1520 deletion affects genes other than that encoding E1B 55K; it also deletes a coding sequence from part of the *E3* region (*11*). To test whether the restricted host range of dl1520 is specifically due to loss of E1B 55K, we constructed a U2OS cell line that expressed E1B 55K under the control of the human cytomegalovirus immediate-early promoter (*13*). We predicted that this would render the U20S cells susceptible to dl1520 infection. The E1B 55K protein produced in these transfected cells (designated UFL-A) interacted with endogenous p53 protein, as demonstrated by co-immunoprecipitation of E1B 55K with an antibody against p53, and vice versa (Fig. 2). To test whether these cells had become sensitive to dl1520, we infected them with dl1520 or wild-type adenovirus and monitored them for CPE. Staining of cells with crystal violet 5 days after infection with dl1520 revealed little evidence of CPE in control cells transfected with empty vector (U-vec cells), whereas UFL-A cells were completely lysed by dl1520. Wild-type adenovirus lysed U-vec and UFL-A cells with comparable efficiency. These data confirm that the inability of dl1520 to replicate in and kill $p53^+$ cells is due to lack of E1B 55K expression.

To confirm that the attenuation of dl1520 was due to its inability to inactivate p53, we constructed a U2OS cell line that expressed a mutant E1B 55K protein that was incapable of binding and inactivating p53. The p53-binding domain of the E1B 55K protein [residues 215 to 354 of the 55K protein (*5*)] was deleted at the DNA level, and the modified gene was transfected into U20S cells. A cell line (U4.5-H) expressing the mutant protein was isolated and characterized. The mutant E1B protein was expressed efficiently but did not bind to p53 (Fig. 3), and in transactivation assays with a p53-dependent reporter plasmid, significant p53 activity was retained relative to cells expressing empty vector. In CPE assays, these cells were resistant to dl1520. These data suggest that the inability of dl1520 to produce E1B 55K protein capable of binding p53 is responsible for its inability to replicate efficiently in $p53^+$ cells.

Further evidence to support the hypothesis that the host restriction of dl1520 is due to p53 is presented in Fig. 4. RKO human colon carcinoma cell lines have previously been shown to express functional p53, and a derivative has been made (RKO.p53.13) in which p53 function has been ablated by expression of a dominant negative p53 allele (*14*). RKO cells were not killed by dl1520 at multiplicities of infection (MOIs)

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up to one plaque-forming unit (PFU) per cell, whereas the p53-defective line was killed at MOIs of 0.01 PFU per cell. Thus, p53 inactivation increases sensitivity to dl1520 by a factor of about 100.

We next examined the therapeutic efficacy of dl1520 in vivo by growing $p53^-$ C33A cells and $p53^+$ U87 human glioblastoma multiforme cells as tumor xenografts in athymic mice (U20S cells do not grow as tumors in nude mice). The tumor cells were injected subcutaneously into each flank of each mouse, and after establishment of palpable tumors (mean tumor volume 150 μl), the tumors were directly injected with CsCl-purified wild-type adenovirus, with dl1520, or with ultraviolet (UV)-inactivated wild-type virus as a negative control every other day for three total doses. Tumor growth was then followed for 6 weeks, at which time the mean tumor volume in each group was determined. Treatment of C33A tumors with dl1520 resulted in an 84% reduction in mean tumor volume as compared with wild-type UV-inactivated adenovirus (unpaired two-tailed test: $P = 0.02$; Fig. 5A). One of the tumors treated with dl1520 underwent complete regression. Wild-type adenovirus was slightly more effective in reducing tumor volume (94% inhibition) at an equivalent dose. In contrast, dl1520-injected $p53^+$ U87 tumors were comparable in size to control-injected tumors after 6 weeks, although shrinkage was seen in some cases. Wild-type adenovirus caused significant tumor inhibition (64% inhibition, $P = 0.05$; Fig. 5B). In another experiment, C33A tumor xenografts were injected with dl1520 or buffer control each day for five consecutive days, and the tumor volumes were calculated weekly. The dl1520-treated tumors were significantly inhibited in their growth as compared with buffer controls (Fig. 5C). Of five treated tumors, three showed a complete regression and one showed a partial re-

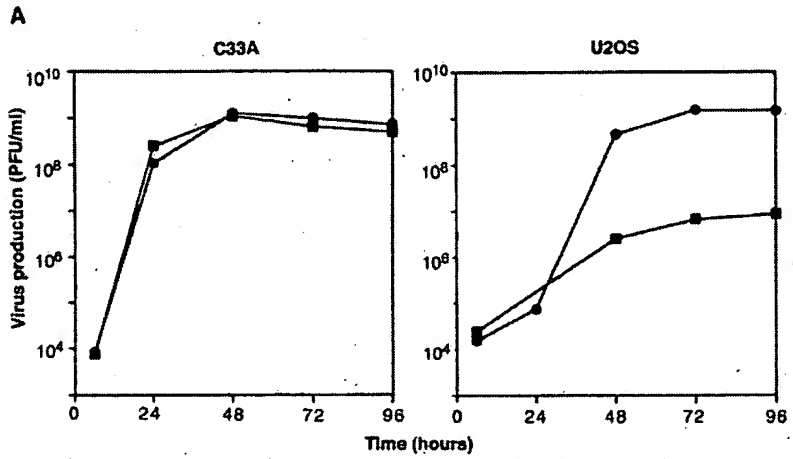

Fig. 1. (**A**) Replication efficiency of dl1520 (squares) and wild-type adenovirus (WT Ad) (circles) in $p53^-$ or $p53^+$ cells. C33A or U2OS cells were infected at a MOI of 1.0 PFU per cell with either WT Ad or dl1520, and virus production was measured by plaque assay (*18*). Data are means of duplicate determinations. (**B**) Selective lysis of $p53^-$ cells by dl1520. Monolayers of C33A cells, HS68 normal diploid fibroblasts, and U2OS cells were infected at a MOI of 10 PFU per cell with either dl1520 (left) or WT Ad (right). After 9 days, viable cells were stained with crystal violet. Uninfected C33A cells were used for the mock infection.

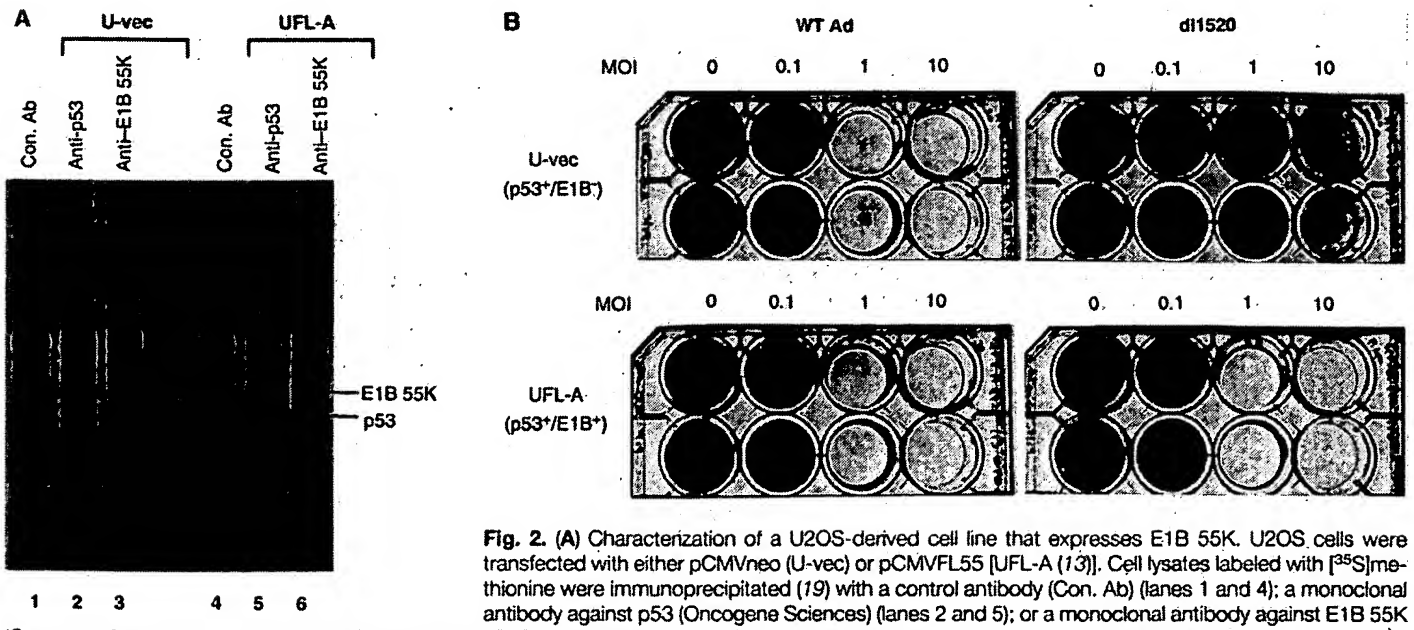

Fig. 2. (**A**) Characterization of a U2OS-derived cell line that expresses E1B 55K. U2OS cells were transfected with either pCMVneo (U-vec) or pCMVFL55 [UFL-A (*13*)]. Cell lysates labeled with [^{35}S]methionine were immunoprecipitated (*19*) with a control antibody (Con. Ab) (lanes 1 and 4); a monoclonal antibody against p53 (Oncogene Sciences) (lanes 2 and 5); or a monoclonal antibody against E1B 55K (Oncogene Sciences) (lanes 3 and 6). (**B**) Expression of E1B 55K renders $p53^+$ U2OS cells sensitive to dl1520. U-vec cells (top plates) and UFL-A cells (bottom plates) were infected with either WT Ad (left) or dl1520 (right) at MOIs of 0, 0.1, 1, or 10. Five days after infection, the plates were stained with crystal violet.

sponse. Tumors responding completely have been followed for over 3 months without evidence of regrowth. In three additional experiments, 12 of 18 C33A tumors injected with 10^8 PFU of dl1520 have shown complete regression (*10*).

To ensure that the reduction of C33A tumor mass was due to dl1520 virus replication, we analyzed sections of the excised tumor for Ad5 capsid proteins. The clear immunohistochemical staining of these sections with antibodies to Ad5 hexon protein (Fig. 6) indicates that dl1520 replicated and disseminated throughout the C33A tumors. No staining was seen in dl1520-treated U87 tumors.

Loss of function of the p53 tumor suppressor gene is the most common genetic defect in human malignancies, affecting more than 50% of all tumors. p53 is thought to monitor the integrity of the

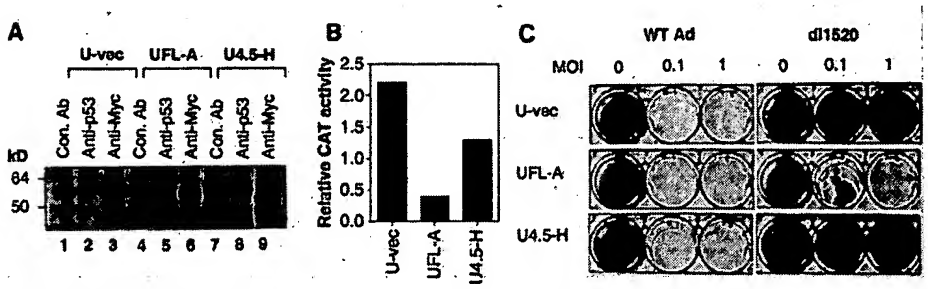

Fig. 3. (**A**) Expression of a mutant E1B 55K protein that does not bind to p53. U-vec, UFL-A, and U4.5-H cells (*13*) were labeled with [^{35}S]methionine and lysates were immunoprecipitated (*19*) with a control antibody (Con. Ab; lanes 1, 4, and 7); a monoclonal antibody against p53 (Oncogene Sciences) (lanes 2, 5, and 8); or a monoclonal antibody against the Myc epitope (lanes 3, 6, and 9). (**B**) The mutant E1B 55K protein produced by U4.5-H cells does not inhibit p53-dependent transactivation of transcription. U-vec, UFL-A, and U4.5-H cells were transfected with 1 μg of pCMVluc and 5 μg of pCOSX1CAT (*20*). CAT activity was normalized to the luciferase activity. (**C**) An E1B 55K mutant incapable of binding p53 does not render p53$^+$ cells sensitive to dl1520. U-vec, UFL-A, and U4.5-H monolayers were infected with either WT Ad (left) or dl1520 (right) at MOIs of 0, 0.1, or 1. Six days after infection, viable cells were stained with crystal violet.

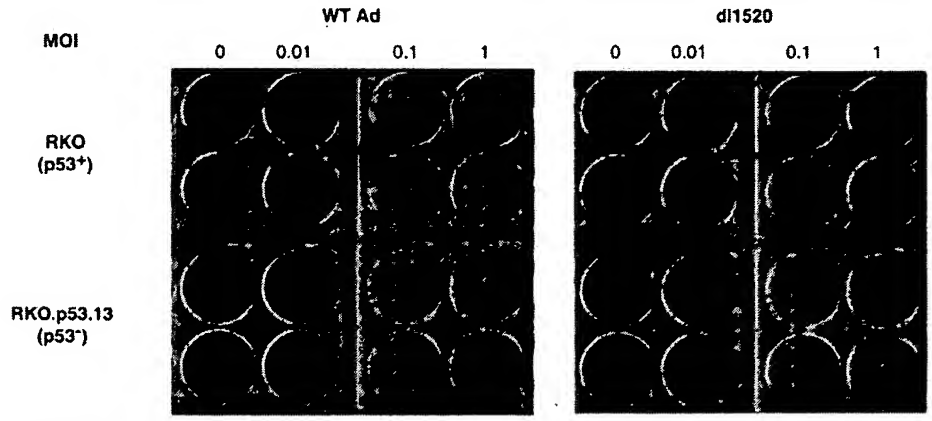

Fig. 4. Effects of dl1520 and WT Ad on RKO cells and RKO.p53.13 cells lacking functional p53. Cells were infected at the MOIs shown and stained for viability 8 days later.

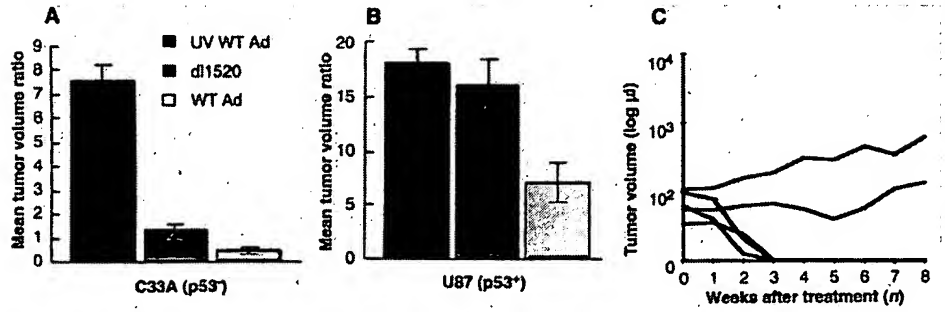

Fig. 5. Effects of UV-inactivated WT Ad, dl1520, or WT Ad on p53$^-$ and p53$^+$ human tumor xenograft growth. (**A**) C33A cells (p53$^-$) or (**B**) U87 human glioblastoma multiforme cells (p53$^+$) were injected subcutaneously into the flanks of *nu/nu* mice. Five weeks later, the tumor volume ratio was calculated (*21*). (**C**) C33A cells were injected subcutaneously as above. Once tumors reached about 80 μl in volume, they were injected with 10^8 PFU of dl1520 (solid lines) or buffer (dashed lines) for 5 days consecutively. The daily dose was divided equally into each tumor quadrant (15 μl per quadrant).

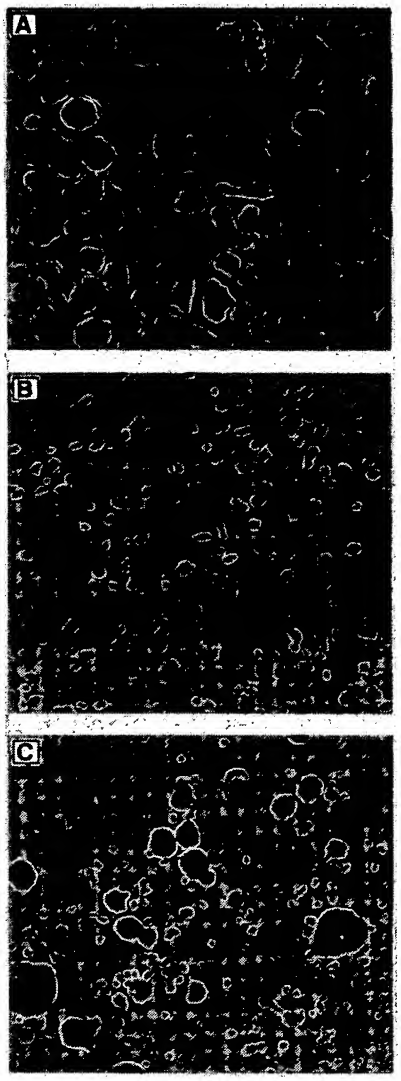

Fig. 6. Immunohistochemical staining of adenovirus hexon protein in human tumor xenografts treated with dl1520 or WT Ad. p53$^-$ C33A tumors and p53$^+$ U87 tumors were excised from nude mice 5 weeks after treatment with dl1520 or WT Ad. Tumors were immunostained with an antibody specific for adenovirus hexon protein (*22*). Cells containing the structural hexon protein, encoded by adenovirus late genes, are stained brown, indicating viral replication. (**A**) p53$^-$ C33A tumor treated with dl1520, showing positive staining in viable tumor cells. (**B**) p53$^+$ U87 tumor after treatment with dl1520, showing absence of hexon protein staining. (**C**) U87 tumor treated with WT Ad, showing numerous tumor cells positively stained for adenovirus hexon protein.

cellular genome and responds to DNA damage by inducing cell cycle arrest or apoptosis. Tumors lacking p53 are unable to mount these responses and therefore respond poorly to radiation or chemotherapy. Thus, a therapeutic strategy that allows selective killing of p53-deficient cells would be of great value.

The strategy presented here takes advantage of the p53 defect in human cancer cells to complement the growth of an adenovirus mutant that has a specific replication deficiency. Our results with cultured cells and tumor-bearing mice show that an adenovirus lacking the E1B 55K gene product replicates in and lyses tumor cells deficient in p53 but replicates 100 times less efficiently in cells expressing functional p53. Recently, it was reported that the orf6 region of adenovirus E4 can participate in p53 inactivation (*15*). The relative contributions of E1B 55K and E4orf6 toward p53 inactivation are currently being investigated.

Our results suggest that adenoviruses with host ranges restricted to tumor cells may be useful in treating human cancers. Several issues remain to be addressed. First is the degree to which the host immune response may affect virus spread and cell killing. Unfortunately, the host range of human adenoviruses is restricted, and no appropriate animal models exist to allow further exploration of this issue. However, the replication of dl1520 is restricted to tumor cells: An immune response directed at late viral antigens expressed on the surface of infected tumor cells might augment tumor killing in an immunocompetent host. In addition, the intratumoral recruitment and stimulation of tumor-specific T lymphocytes theoretically could lead to systemic antitumor immunity. Second, the ability of the virus to spread to distant sites and to infect metastatic tumor cells needs to be addressed because direct intratumoral injection limits the potential benefit of this approach to accessible tumors (primary brain tumors and cancers of the head and neck, for example). However, the selectivity and potency of this virus suggest that this approach should be tested in the clinic. Because of these data as well as data showing that dl1520 is not toxic in mice or cotton rats at doses up to 10^9 PFU (*16*), dl1520 is being tested in Phase I trials in patients with $p53^-$ squamous cell carcinoma of the head and neck (*17*).

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13. A Myc epitope tag was added at the DNA level to the NH_2-terminus of E1B 55K, and the construct was subcloned into pCMVneo. This clone was designated pCMVFL55. Another construct was made in which amino acids 216 through 354 of the Myc-tagged full-length E1B 55K were deleted. This clone, which was expressed in U4.5H cells, was designated pCMV55D4.5. The inserts of pCMVFL55k and pCMV55D4.5 were sequenced to confirm that they were correct (*9*).
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16. Twenty cotton rats (the cotton rat is a permissive host for human adenovirus) were given intraperitoneal injections of up to 10^9 PFU of dl1520 per animal. No evidence of toxicity was seen in these animals, as assayed by animal weight gain, serum chemistries, and organ histopathology (including the liver, lung, kidneys, heart, gastrointestinal tract, and brain).
17. Phase I tests of dl1520 (ONYX-015) began in April 1996 at the University of Texas, San Antonio (by Daniel Von Hoff) and the Beatson Institute, Glasgow, Scotland (by Stanley Kaye).
18. Plates were scraped into 1 ml of media and frozen. Lysates were prepared by three cycles of freezing and thawing, followed by a 30-s pulse in a sonicator water bath. Serial dilutions of the lysates were titered on HEK293 cells (human embryonic kidney cells expressing the *E1* region of Ad2).
19. Cells were harvested after being incubated in media containing 200 μCi of [^{35}S]methionine for 2 hours at 37°C. The cells were lysed in 50 mM Hepes (pH 7.9), 250 mM NaCl, 50 mM NaF, 5 mM EDTA, 0.1 mM Na orthovanadate, and 0.1% Triton X-100. The protein concentration was determined by Bradford analysis. Equal amounts of protein were immunoprecipitated with the appropriate antibody for 1 hour at 4°C, protein G–Sepharose (Sigma) was added, and the samples were incubated for an additional 30 to 60 min at 4°C. The immunocomplexes were washed three times in lysis buffer, resuspended in SDS sample buffer, and resolved on 10% polyacrylamide gels. The gels were fixed, dried, and subjected to autoradiography.
20. Lysates were prepared 36 hours after transfection and chloramphenicol acetyl transferase (CAT) (Boehringer-Mannheim), and luciferase (Promega) assays were performed according to the manufacturer's instructions.
21. C33A human cervical carcinoma and U87 glioblastoma multiforme cells were obtained from the American Type Culture Collection. Female athymic *nu/nu* mice were obtained from the Harlan Sprague-Dawley Company at 4 to 6 weeks of age and were quarantined for at least 2 weeks before the study. Animal experiments were carried out in accordance with both institutional and federal animal care regulations. U87 and C33A cells were grown in Dulbecco's modified Eagle's medium (DMEM) with high glucose and supplemented with 10% fetal bovine serum, nonessential amino acids, L-glutamine, and penicillin and streptomycin until confluence. Cells were harvested through two consecutive trypsinizations, centrifuged at 300*g* for 8 min, washed twice, and resuspended in serum-free DMEM, and the cell density was adjusted to 5×10^7 cells/ml. 1.0×10^7 cells in 0.2 ml were injected subcutaneously into each flank of 7- to 10-week-old female nude mice. Tumor volumes were estimated with the following formula: (maximal length) × (perpendicular width)^2/2. Once tumors reached a mean size of 110 to 150 μl, animals were directly injected with 10^9 PFU of dl1520 (n = 10 U87 tumors and 5 C33A tumors), Ad2 (n = 10 tumors), or UV-inactivated Ad2 (n = 6 tumors) divided equally into four tumor quadrants (15 μl per quadrant) every other day for three total doses. Tumor volumes were recorded weekly until termination of the study. At the time of termination, the tumor volume ratio was calculated as follows: (tumor volume at study termination)/(tumor volume at the time of virus injection). The unpaired t test (two-tailed) was used to compare final tumor volume ratios in various groups.
22. Immunohistochemistry was performed on formalin-fixed paraffin-embedded tumors that had been cut into 4-μm sections, hydrated, and digested with pronase. The primary antibody (MAB805, Chemicon International) is specific for all 41 serotypes of adenovirus hexon protein. Tissues were incubated for 1 hour at 35°C with an antibody dilution of 1:1000. A biotinylated goat secondary antibody to mouse immunoglobulin was then applied, followed by a streptavidin–horseradish peroxidase conjugate. Diaminobenzidine was used as the chromagen, and slides were counterstained with hematoxylin.

23. We thank A. Berk (University of California, Los Angeles) for providing wild-type adenovirus and dl1520; E. White (Rutgers University) for a cDNA encoding full-length E1B 55K polypeptide; M. Kastan (Johns Hopkins University) for RKO cells; J. Hassell (McMaster University) for HEK293 cells; D. Von Moff, G. Mangold, and D. Dexter (San Antonio Cancer Treatment and Research Center) for help with animal models; J. Olesch for technical help; and C. Maack for many stimulating discussions.

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